# Within-lineage variability of ST131 Escherichia coli isolates from humans and companion animals in the South of Europe

Pomba, C.; López-Cerero, L.; Bellido, M.; Serrano, L.; Belas, A.; Couto, N.; Cavaco-Silva, P.; Rodríguez-Bano, J.; Pascual, A.

*Published in:*
Journal of Antimicrobial Chemotherapy

*DOI:*
10.1093/jac/dkt343

separated by 22 bp instead of 17 bp (see Figure S1, available as Supplementary data at *JAC* Online). An additional promoter was predicted by Softberry BPROM promoter prediction and consisted of the −35 box of IS*18* and a −10 box separated by 13 bp (Figure S1, available as Supplementary data at *JAC* Online).[10] Interestingly, the TTCAAT −35 box identical to that from Baz ($bla_{OXA-228}$) was adjacent to the left inverted repeat.

OXA-228-like expression in isolate KH243 was compared with that in carbapenem-susceptible *A. bereziniae* isolate G3-59 by semi-quantitative RT–PCR (qRT–PCR) using *rpoB* as the reference gene. The primers used for qRT–PCR are shown in Table 1. Three independent experiments were performed using freshly prepared RNA and cDNA and revealed a 56-fold (±3.84) overexpression of $bla_{OXA-228-like}$ in isolate KH243 compared with that in G3-59. To investigate the potential to mediate carbapenem resistance, IS*18*::$bla_{OXA-257}$ was cloned into the shuttle vector pWH1266, but we were unable to transfer this into *A. bereziniae* G3-59. However, the construct was successfully transferred into *A. baumannii* ATCC 17978 by electroporation, as previously described for *Pseudomonas aeruginosa*.[11] Expression of $bla_{OXA-257}$ in *A. baumannii* ATCC 17978 raised both imipenem and meropenem MICs from 0.25 to >32 mg/L, demonstrating that IS*18*::$bla_{OXA-257}$ is able to confer carbapenem resistance.

In conclusion, this study has detected an IS upstream of the intrinsic $bla_{OXA}$ in *A. bereziniae*, a phenomenon that has not been described in this species so far. IS*18* conferred overexpression of OXA-257, which mediated carbapenem resistance in *A. bereziniae* and *A. baumannii*. Moreover, because IS*18* has previously been described adjacent to acquired $bla_{OXA}$ in *A. baumannii*,[8] these data suggest a potential for dissemination of OXA-257 in the genus *Acinetobacter*.

## Funding

This work was supported by grants from Bundesministerium für Bildung und Forschung (BMBF), Germany, Klinische Forschergruppe Infektiologie (grant number 01 KI 0771 to P. G. H and H. S.). E. Z. and H. S. were supported by Magic Bullet. Magic Bullet is a project funded by the European Union—Directorate General for Research and Innovation through the Seventh Framework Program for Research and Development (Grant Agreement 278232) and has been running since the 1 January 2012 (duration 36 months).

## Transparency declarations

None to declare.

## Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

## References

**1** Nemec A, Musilek M, Sedo O *et al*. *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. *Int J Syst Evol Microbiol* 2010; **60**: 896–903.

**2** Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 2007; **5**: 939–51.

**3** Bonnin RA, Ocampo-Sosa AA, Poirel L *et al*. Biochemical and genetic characterization of carbapenem-hydrolyzing β-lactamase OXA-229 from *Acinetobacter bereziniae*. *Antimicrob Agents Chemother* 2012; **56**: 3923–7.

**4** Gundi VA, Dijkshoorn L, Burignat S *et al*. Validation of partial *rpoB* gene sequence analysis for the identification of clinically important and emerging *Acinetobacter* species. *Microbiology* 2009; **155**: 2333–41.

**5** Higgins PG, Perez-Llarena FJ, Zander E *et al*. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2013; **57**: 2121–6.

**6** Woodford N, Ellington MJ, Coelho JM *et al*. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006; **27**: 351–3.

**7** Turton JF, Ward ME, Woodford N *et al*. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006; **258**: 72–7.

**8** Villalon P, Valdezate S, Medina-Pascual MJ *et al*. Epidemiology of the *Acinetobacter*-derived cephalosporinase, carbapenem-hydrolysing oxacillinase and metallo-β-lactamase genes, and of common insertion sequences, in epidemic clones of *Acinetobacter baumannii* from Spain. *J Antimicrob Chemother* 2013; **68**: 550–3.

**9** Higgins PG, Zander E, Seifert H. Identification of a novel insertion sequence element associated with carbapenem resistance and the development of fluoroquinolone resistance in *Acinetobacter radioresistens*. *J Antimicrob Chemother* 2013; **68**: 720–2.

**10** Rudant E, Courvalin P, Lambert T. Characterization of IS*18*, an element capable of activating the silent *aac(6′)-Ij* gene of *Acinetobacter* sp. 13 strain BM2716 by transposition. *Antimicrob Agents Chemother* 1998; **42**: 2759–61.

**11** Choi KH, Kumar A, Schweizer HP. A 10 min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: application for DNA fragment transfer between chromosomes and plasmid transformation. *J Microbiol Methods* 2006; **64**: 391–7.

*J Antimicrob Chemother* 2014
doi:10.1093/jac/dkt343
Advance Access publication 10 September 2013

# Within-lineage variability of ST131 *Escherichia coli* isolates from humans and companion animals in the south of Europe

**C. Pomba[1], L. López-Cerero[2]*, M. Bellido[2], L. Serrano[3], A. Belas[1], N. Couto[1], P. Cavaco-Silva[4,5], J. Rodríguez-Baño[2] and A. Pascual[2,3]**

*[1]CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Lisbon, Portugal; [2]University Hospital Virgen Macarena, Seville, Spain; [3]School of Medicine, University of Seville, Seville, Spain; [4]CIIEM, Instituto Superior de Saúde Egas Moniz, Monte da Caparica, Portugal; [5]Technophage, SA, Lisbon, Portugal*

*Corresponding author. E-mail: llopez@us.es

**Keywords:** pulsotypes, fluoroquinolone resistance, ESBLs, MLST, PFGE

Sir,

A multiresistant CTX-M-15-producing clonal group of *Escherichia coli* isolates belonging to phylogroup B2, namely O25b:H4/ST131, has recently emerged and spread across three continents; it predominantly causes community-onset infections in humans and to a lesser extent in dogs and cats.[1–3] Some specific lineage variations within this clone, detected by PFGE, such as pulsotype 968, have been associated with clonal commonality across pets and humans.[4,5] Important comparative studies to date have only included a small number of isolates from companion animals.

A total of 148 ST131 isolates, identified by screening unselected sequential isolates, were compiled in two different studies carried out in Lisbon ($n=59$) and Seville ($n=89$). The veterinary *E. coli* isolates ($n=31$) were collected during 2004–09 from dogs and cats with a urinary tract infection (UTI). Portuguese human UTI clinical isolates ($n=28$) were obtained during 2005–06 in one hospital and in a community diagnostic laboratory covering an area of 365 000 inhabitants in the Lisbon region. ST131 human isolates from Seville were collected from a systematic prospective study in which all *E. coli* isolates from two hospitals covering an area with 1 million inhabitants were screened for O25b serogroup during 30 weeks in 2010. Isolates from Seville were mainly recovered from UTI (98%).[6]

The ST131 clone was screened by PCR with specific primers for O25b *rfb*, allele 3 of the *pabB* gene and B2 genetic traits, and by multiplex PCR for phylogroup B2 typing using two different combinations of primers.[7,8] Susceptibility testing was performed using commercial microdilution plates and the disc diffusion method for nalidixic acid, ciprofloxacin, amoxicillin/clavulanic acid, gentamicin, tobramycin, amikacin, trimethoprim/sulfamethoxazole and fosfomycin. Extended-spectrum β-lactamase (ESBL) production was screened for by the double-disc synergy test, and ESBL typing was determined by PCR and further sequencing.[2] PFGE analysis was carried out according to the PulseNet protocol in both laboratories. *Salmonella* serotype Braenderup strain H9812 was used for normalization and dendrograms were created with Fingerprinting 3.0 software (Bio-Rad), using the Dice coefficient.

In this collection of ST131 isolates from the B2 phylogenetic group, the overall prevalence of ESBL producers was 41% ($n=60$). Fifty-six (93%) harboured CTX-M-15, three (5%) CTX-M-32 and one (2%) CTX-M-14. Higher rates of ESBL production were observed in the human-derived isolates than in the pet-derived isolates: 57 ESBL-producing human isolates out of 117 (49%) versus three ESBL-producing animal isolates out of 31 (10%). Human ST131 isolates were found to be more resistant to ciprofloxacin, trimethoprim/sulfamethoxazole and tobramycin than isolates from pets ($P<0.001$). With respect to the ESBL-producing isolates examined

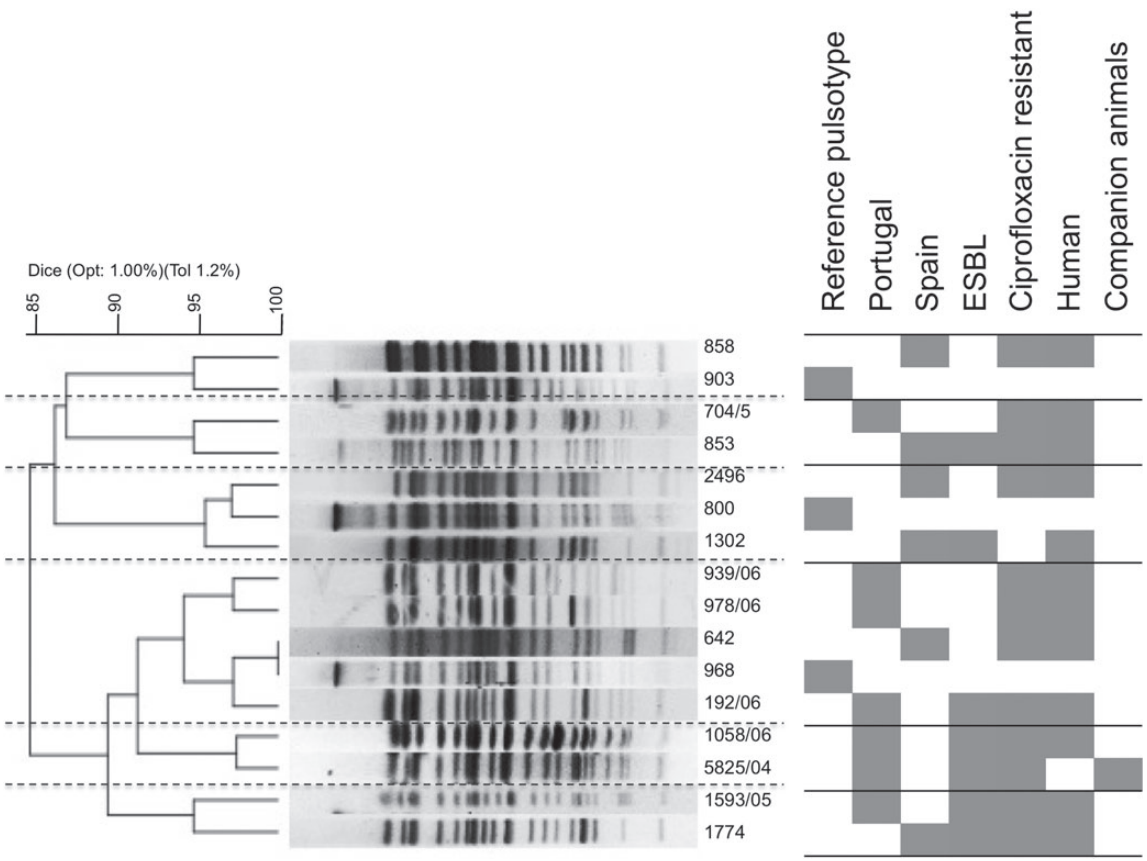


**Figure 1.** Dendogram showing the six ST131 *E. coli* clusters based on the XbaI-generated profile (Dice similarity value ≥94%, black line), which included pulsotypes common between Spain and Portugal, common between human and pets, and matching with international pulsotypes. Grey squares, positive trait; white squares, negative trait.

in this study, most harboured CTX-M-15, as was universally observed in previous surveys of humans and companion animals.[1,5,9] Fosfomycin resistance was higher in Portugal than in Seville among human-derived isolates ($P<0.001$) and, in contrast with previous reports from Spain,[10] this resistance trait has arisen independently in different human pulsotypes.

A total of 95 pulsotypes were distinguished (see Figure S1, available as Supplementary data at *JAC* Online). Three clusters (7% of human isolates) included isolates from Seville and Lisbon. Only one cluster containing one human and one dog isolate was detected. Seven (6%) human isolates exhibited a close genetic relationship with international PFGE profiles: four isolates from Seville matched type 903, and a cluster containing three human isolates from Lisbon and one from Seville matched type 968 (Figure 1). An additional Spanish isolate showed a similar profile to pulsotype 800. Isolates clustering with pulsotype 968 were all resistant to ciprofloxacin, three out of four were intermediately resistant or resistant to fosfomycin and one was a CTX-M-15 producer.

This study confirmed that some predominant lineages within the ST131 clone, mainly pulsotype 968, are present and have spread in Portugal and Spain. However, common patterns between these two countries accounted for less than 10% of all isolates, probably because of the different time frame of the collections from Portugal (2004–09) and Spain (2010). This is the first report of pulsotype 968 in Spain. The 968 pulsotype had been previously detected among human ST131 isolates in Portugal.[4,11] In our survey, this transboundary 968 type was exclusively observed among human infection isolates, in contrast with previous studies where it was associated with pets.[4,11] The 968 type belongs to the *fimH*30 type, which was significantly associated with fluoroquinolone resistance.[12]

Only 3% of the Portuguese group contained pulsotypes that were common between humans and pets. Our results agree with the conclusions of a large international comparison of isolates carried out by Johnson *et al.*,[4] which argued against pet–human commonality for ST131 isolates. None of the animal isolates could be associated with the prevalent pulsotypes. Isolates from companion animals were also less clustered and represented single pulsotypes, which may suggest less dissemination among pets.

The present work demonstrates the presence of high-prevalence international pulsotypes in two different countries in the south of Europe. However, the study has some limitations, in terms of the small number of cases in some groups and the differences in time frame between the areas. Nonetheless, variability among *E. coli* ST131 isolates at the pulsotype level was country-specific with little exchange between Seville and Lisbon and between humans and companion animals. In this study, the *E. coli* ST131 within-lineage genetic variation that was found argues in favour of a rapid host species adaptation and an ongoing dissemination of this antimicrobial drug-resistant pathogen.

## Acknowledgements

We thank James R. Johnson for kindly providing 15 isolates representing high-prevalence ST131 pulsotypes.

## Funding

This work was co-funded by FEDER funds through the Programa Operacional Factores de Competitividade – COMPETE and by National funds through the FCT – Fundação para a Ciência e a Tecnologia, Project PEst-OE/AGR/UI0276/2011, the Ministerio de Ciencia e Innovacion (Instituto de Salud Carlos III, PI10/01955), Junta de Andalucia (PI0034-2009, P09-CTS 5259) and co-financed by the European Development Regional Fund 'A way to achieve Europe' ERDF, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015).

## Transparency declarations

None to declare.

## Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

## References

**1** Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al*. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; **61**: 273–81.

**2** Díaz MA, Hernández-Bello JR, Rodríguez-Baño J *et al*. Diversity of *Escherichia coli* strains producing extended-spectrum β-lactamases in Spain: second nationwide study. *J Clin Microbiol* 2010; **48**: 2840–5.

**3** Johnson JR, Miller S, Johnston B *et al*. Sharing of *Escherichia coli* sequence type ST131 and other multidrug-resistant and urovirulent *E. coli* strains among dogs and cats within a household. *J Clin Microbiol* 2009; **47**: 3721–5.

**4** Johnson JR, Nicolas-Chanoine MH, DebRoy C *et al*. Comparison of *Escherichia coli* ST131 pulsotypes, by epidemiologic traits, 1967–2009. *Emerg Infect Dis* 2012; **8**: 598–607.

**5** Platell JL, Johnson JR, Cobbol RN *et al*. Multidrug-resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST131 in animals and foods. *Vet Microbiol* 2011, **153**: 99–108.

**6** López-Cerero L, Bellido MM, Serrano L *et al*. *Escherichia coli* O25b:H4/ST131 are prevalent in Spain and are often not associated with ESBL or quinolone resistance. *Enferm Infecc Microbiol Clin* 2013; **31**: 385–8.

**7** Clermont O, Dhanji H, Upton M *et al*. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. *J Antimicrob Chemother* 2009; **64**: 274–7.

**8** Doumith M, Day MJ, Hope R *et al*. Improved multiplex PCR strategy for rapid assignment of the four major *Escherichia coli* phylogenetic groups. *J Clin Microbiol* 2012; **50**: 3108–10.

**9** Ewers C, Grobbel M, Stamm I *et al*. Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-β-lactamase-producing *Escherichia coli* among companion animals. *J Antimicrob Chemother* 2010; **65**: 651–60.

**10** Oteo J, Orden B, Bautista V *et al*. CTX-M-15-producing urinary *Escherichia coli* O25b-ST131-phylogroup B2 has acquired resistance to fosfomycin. *J Antimicrob Chemother* 2009; **64**: 712–7.

**11** Platell JL, Cobbold RN, Johnson JR *et al*. Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. *Antimicrob Agents Chemother* 2011; **55**: 3782–7.

**12** Johnson JR, Tchesnokova V, Johnston B *et al*. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. *J Infect Dis* 2013; **207**: 919–28.